LOST LAMBS

Lessons Learned From Scrapie Outbreak

Katherine O'Rourke isn't the kind of doctor that normally makes house calls. But 2001 was different. That's when O'Rourke, an ARS microbiologist with a Ph.D in veterinary science and immunology, heard about an interesting case in Tucson, Arizona.

It involved a nervous system disease called scrapie that had befallen a flock of about 36 Suffolk sheep managed by José A. Bernal, a science teacher at Amphitheater High School in downtown Tucson. Bernal housed the flock at the school's nearby "lamb lab," a facility where students could gain hands-on experience raising the animals for market and learn about science and agriculture.

But the course became a tough lesson in loss, starting in 1997 when scrapie claimed its first victim: "Baby Face," a prized, 7-year-old pet ewe that Bernal had raised from a lamb. Later, more flock members tested positive for the disease—also known as ovine transmissible spongiform encephalopathy—and, by law, had to be destroyed.

"We ended up getting rid of every single ewe we had that was at risk," says Bernal. "It was bad." So much so, the lamb lab nearly faced closure in 2001. His problems weren't unique, though. By September of that year, 98 other U.S. cases of scrapie had been reported in sheep and 7 in goats. Yearly scrapie losses cost American sheep and goat producers an estimated $20 to $25 million.

Through Bret A. Combs, with APHIS Veterinary Services (VS), Bernal contacted O'Rourke, who was leading a study to decipher the genetic underpinnings of scrapie resistance in sheep at ARS' Animal Disease Research Unit, and Washington State University, both located in Pullman, Washington. There, O'Rourke had also helped pioneer development of a so-called third-eyelid test to detect scrapie's main causative agent: a malformed protein called a prion.

By combining this new, live-animal testing method with sanitation, genetics, and other measures, O'Rourke felt it would be possible to eliminate scrapie from the students' flock. But first she needed to make a house call to better assess the situation. So, in March 2001, she booked a flight to Tucson to meet with Bernal and his students. John V. Duncan, an APHIS-VS collaborator from Casper, Wyoming, went too.

Science in the Classroom

After arriving, says O'Rourke, "We went to the lamb lab and did our live-animal and genotyping tests on the sheep." Armed with the results, she and Duncan later worked up a genetics-based strategy by which Bernal's class could repopulate their flock and eventually certify it as scrapie free.

Future Farmers of America students from Amphitheater High School, Tucson, Arizona, lead sheep from their pens to a site where the animals will be tested for scrapie.

STEPHEN AUSMUS (K10088-1)

For Bernal, it was a golden teaching opportunity, despite the stress of losing several sheep and the lamb lab's near closure. "Our kids had the opportunity to learn firsthand about scrapie and how you go about identifying diseases," says Bernal, who has worked on a cattle ranch and studied animal science in college. "I always want my kids to work with people like O'Rourke who are on the cutting-edge of science," he adds.

One example of this was O'Rourke's use of the third-eyelid test. It's a relatively noninvasive procedure that can detect scrapie-causing prions in young sheep before clinical signs of

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APHIS veterinarian John Duncan (from Wyoming) clips a tiny piece of a third eyelid from a sheep while students and animal behavior teacher Jose Bernal (wearing striped shirt) hold the sheep.

the disease appear. Around 3 years of age, an infected animal may experience trembling, lip smacking, erratic behavior, and weight loss. Eventually, it becomes so sick it must be destroyed. Through working with Wyoming's commercial wool producers, Duncan and a coalition of federal, state, and university veterinarians were able to extensively field-test the procedure and show its usefulness in screening flocks for scrapie before these clinical signs appear. Their study was published in the journal *Clinical and Diagnostic Laboratory Immunology*.

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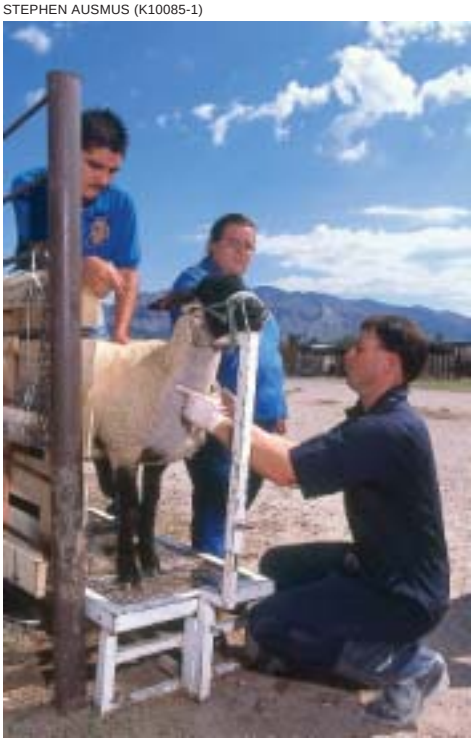

William Rivera and Betty Masulis keep a sheep calm while veterinarian John Duncan draws blood to test for genetic resistance to scrapie.

Fine-tuning Flocks With Genetics

This May, Katherine O'Rourke began using genetic testing to an even greater degree in an ARS-funded project led by Robert H. Stobart, along with Gary Moss and Bill Russell at the University of Wyoming's Department of Animal Sciences in Laramie. There, they are evaluating two flocks of sheep representing four breeds—Suffolk, Columbia, Hampshire, and Rambouillet—for traits of economic importance to producers. These include fiber diameter and staple length for the wool breeds, and meat production, weight gain, number of lambs born and weaned, and weaning weights for all the breeds. The researchers will also evaluate lamb performance from weaning to slaughter.

JACK DYKINGA (K8829-1)

Microbiologist Katherine O'Rourke prepares a sample of sheep eyelid tissue for a scrapie test.

The idea is to help producers select sheep with both low susceptibility to scrapie and traits that will turn a profit. This, too, will become increasingly important in the next 10 years as Wyoming and other states and USDA seek to eradicate scrapie, which has hurt U.S. exports of breeder stock, frozen semen, bone meal, and other sheep-derived products.

Meantime, the group plans to compare their project's results with those of Irish researchers who are running a similar study under different conditions. That way, O'Rourke explains, "we get the expertise of another laboratory studying this important question, and we get additional statistical evaluation of the results."

And if time permits, she hopes to make another house call to the lamb lab.—By **Jan Suszkiw,** ARS.

Previously, veterinarians confirmed scrapie by examining tonsil or brain tissue from sheep that had died or been destroyed to prevent them from infecting healthy animals. But with the eyelid test, all that's needed is a small sample of lymph tissue snipped from a special membrane covering the sheep's eye, called the third eyelid. Prions collect on this third eyelid, explains O'Rourke. She helped design a monoclonal antibody that binds to the malformed protein so that it can be identified.

A Primer in Genetics

Genetic testing works differently. Instead of using an antibody to find a protein antigen, this method uses polymerase chain reaction (PCR) and other molecular technologies to home in on a specific gene of interest. In the case of the students' flock, O'Rourke tested for three variations of the gene that codes for the prion protein: 171QQ, 171HH, or 171HQ. Each variant predisposes a sheep to scrapie.

"We break apart, or lyse, white blood cell samples with detergent and then remove their DNA," O'Rourke explains. "We use PCR to locate the prion gene and then use an enzyme and primers to replicate the DNA region in which the gene resides.

Once the gene segment is reproduced, it is labeled with chemical "letters" called nucleotides. The result is a complementary sequence of letters that serves as the prion protein's calling card—and the sheep's susceptibility to scrapie.

In Baby Face, the gene variants 171QQ or 171HQ probably predisposed her to the disease by enabling the prion protein to replicate in her brain and lymph tissues. "A sheep might be genetically predisposed to scrapie, but the scrapie agent has to come in from the outside," notes O'Rourke. There are different ways Baby Face could have become infected. One possibility is that she grazed an area where an infected ewe had given birth, aborted, or deposited placental material containing the prion.

The prion has a shape-shifting nature that makes it toxic to the animal. "It takes on the look of a pleated sheet rather than a smooth helix," she notes. Some sheep, however, are endowed with beneficial forms of the gene—dubbed 171QR or 171RR—that actually prevent such shape-shifting. "We know that the 171R variant works, but we don't know how," says O'Rourke. Researchers are still debating whether or not to use the term "resistant" to describe such animals.

Either way, "it's a lucky break for sheep that have this gene," O'Rourke adds. But only about half of any given flock is likely to harbor the 171QR/RR variant, she adds. With genetic testing, though, producers may soon be able to tip the scales in their flock's favor by checking for the protective gene in rams used for breeding.

So far, the strategy seems to be working for Bernal's class. Their flock now boasts 35 ewes and 2 rams and, as of March, the sheep have been deemed scrapie free by inspectors.

Once again, Bernal's class plans on showing their sheep at the state fair, and he has high hopes they'll become Arizona's "first scrapie-free flock with a protocol in place" for preventing the disease.

"The whole thing is a great story," says O'Rourke of the students' efforts. "It's also a small-scale example of what's going on around the country to control scrapie."—By **Jan Suszkiw,** ARS.

This research is part of Animal Production, Product Value, and Safety, an ARS National Program (#103) described on the World Wide Web at http://www.nps.ars.usda.gov.

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Top: Students watch as APHIS veterinarian John Duncan performs a test for scrapie susceptibility. Future Farmers of America members are wearing blue shirts. Bottom: The group eagerly awaits the test results—samples turning blue indicate susceptibility.